

CLAIMS

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1. A gene having a nucleotide sequence of a nucleic acid selected from the group consisting of:
- 5 (A) a nucleic acid encoding a polypeptide having the amino acid sequence of SEQ ID NO: 14 of the Sequence Listing or a partial sequence thereof, the polypeptide possessing a ceramidase activity;
- (B) a nucleic acid having a nucleotide sequence of SEQ ID NO: 15 of the Sequence Listing or a partial sequence thereof and encoding a polypeptide
10 possessing a ceramidase activity;
- (C) a nucleic acid encoding a polypeptide consisting of an amino acid sequence resulting from deletion, addition, insertion or substitution of at least one amino acid residue in the amino acid sequence of SEQ ID NO: 14 of the Sequence Listing, the polypeptide possessing a ceramidase activity;
- 15 (D) a nucleic acid consisting of a nucleotide sequence resulting from deletion, addition, insertion or substitution of at least one base in the nucleotide sequence of SEQ ID NO: 15 of the Sequence Listing and encoding a polypeptide possessing a ceramidase activity;
- (E) a nucleic acid capable of hybridizing to a complementary strand of a
20 nucleic acid of any one of the above (A) to (D), under stringent conditions, and encoding a polypeptide possessing a ceramidase activity; and
- (F) a nucleic acid having a nucleotide sequence different from the nucleic acid of any one of above (A) to (E) via degeneracy and encoding a polypeptide possessing a ceramidase activity.

2. The gene according to claim 1, wherein the ceramidase activity of the polypeptide is capable of being detected by the following steps:

- (a) incubating a gene expression product in a reaction mixture [composition: 550 pmol of C12-NBD-ceramide and 1.0% (W/V) sodium cholate in 20 μ l of 25 mM Tris-hydrochloric acid buffer (pH 7.5)] at 37°C for 30 minutes, to react the mixture; and
- (b) detecting generation of a C12-NBD-fatty acid in the reaction product.

3. The gene according to claim 1 or 2, wherein the polypeptide exhibits at least the following characteristics:

- (i) action: hydrolyzing ceramide, to generate sphingoid and a fatty acid;
- (ii) substrate specificity: hydrolyzing N-acylsphingosine; but not acting on galactosylceramide, sulfatide, GM1a, and sphingomyelin;
- (iii) optimum pH: being from 7.0 to 8.0; and
- (iv) lowering of the activity not being found when treated in 20 mM Tris-hydrochloric acid (pH 7.5) containing 0.1% polidocanol at 37°C for 24 hours, but the activity lowering by a treatment at 60°C for 1 hour to about 30% of the activity before the treatment.

4. A recombinant DNA comprising the gene of any one of claims 1 to 3.

5. An expression vector for a microorganism, an animal cell or a plant cell, comprising the gene of any one of claims 1 to 3 or the recombinant DNA of claim 4.

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Q2

6. A transformant carrying the expression vector of claim 5.

7. A method for producing a polypeptide possessing a ceramidase activity, characterized by culturing the transformant of claim 6 under conditions appropriate for expression of the ceramidase gene and production of the polypeptide encoded by the gene, and collecting a polypeptide possessing a ceramidase activity from the resulting culture.

8. A polypeptide having the amino acid sequence of SEQ ID NO: 14 of the Sequence Listing or a partial sequence thereof and possessing a ceramidase activity.

9. A polypeptide possessing a ceramidase activity, encoded by the gene of any one of claims 1 to 3.

10. The polypeptide according to claim 8 or 9, wherein the ceramidase activity is capable of being detected by the following steps:

- (a) incubating a gene expression product in a reaction mixture [composition: 550 pmol of C12-NBD-ceramide and 1.0% (W/V) sodium cholate in 20 μ l of 25 mM Tris-hydrochloric acid buffer (pH 7.5)] at 37°C for 30 minutes, to react the mixture; and
- (b) detecting generation of a C12-NBD-fatty acid in the reaction product.

11. An antisense DNA which is complementary to the gene of any one of claims 1 to 3 or a part thereof.

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Sub
a3

Sub
25 a4

12. An antisense RNA which is complementary to the gene of any one of claims 1 to 3 or a part thereof.

5 13. An expression vector comprising the antisense DNA of claim 11.

Sub Q5 14. An oligonucleotide probe or primer, capable of specifically hybridizing to the gene of any one of claims 1 to 3 or a complementary strand thereof.

10 15. An antibody or a fragment thereof, capable of specifically binding to the polypeptide of any one of claims 8 to 10.

15 16. A method for detecting a gene encoding a polypeptide possessing a ceramidase activity, comprising using the oligonucleotide probe or primer of claim 14.

17. A kit for the use in detection of a gene encoding a polypeptide possessing a ceramidase activity, comprising the oligonucleotide probe and/or primer of claim 14.

20 18. A method for detecting a polypeptide possessing a ceramidase activity, comprising using the antibody or a fragment thereof of claim 15.

25 19. A kit for the use in detection of a polypeptide possessing a ceramidase activity, comprising the antibody or a fragment thereof of claim 15.

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20. A method of controlling an amount of ceramide in a cell and/or in a tissue,
characterized by introducing the gene of any one of claims 1 to 3 or an antisense
nucleic acid thereof into the cell and/or the tissue, thereby controlling the amount
of ceramide in the cell and/or in the tissue.

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